Research Journal of Pharmacology and Toxicology 01[05] 2015
www.asdpub.com/index.php/rjpt

Original Article

The acute effect of the antioxidant drug "U-74389G" on mean corpuscular volume levels during hypoxia reoxygenation injury in rats

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Keywords:
Hypoxia,
Mean corpuscular volume,
U-74389G,
Reoxygenation

Abstract

Background: This experimental study examined the effect of the antioxidant drug "U-74389G", on a rat model and particularly in a hypoxia – reoxygenation (HR) protocol. The effects of that molecule were studied hematologically using mean blood corpuscular volume (MCV) levels.
Methods: 40 rats of mean weight 231.875 g were used in the study. MCV levels were measured at 60 min of reoxygenation (groups A and C) and at 120 min of reoxygenation (groups B and D) with administration of the drug U-74389G in groups C and D.
Results: U-74389G administration significantly increased the predicted MCV levels by 2.88%±0.69 (p= 0.0002). Reoxygenation time non-significantly increased the MCV levels by 0.57%±0.83% (p=0.4103). However, U-74389G administration and reoxygenation time together significantly increased the MCV levels by 1.60%±0.43% (p= 0.0005).
Conclusions: U-74389G administration whether it interacted or not with reoxygenation time has significant increasing short – term effect on recovery pathophysiology of MCV values.

1. Introduction

Permanent or transient damage with serious implications on adjacent organs and systems may be due to tissue hypoxia reoxygenation (HR). The use of U-74389G in HR has been a challenge for many years. However, although the progress was significant, several practical questions have not been clarified yet. They include: a) how potent U-74389G should be b) when should it be administered and c) at what optimal dose U-74389G should be administered. The promising effect of U-74389G in tissue protection has been noted in several HR studies. U-74389G or also known as 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-pregna-1,4,9(11)-triene-3,20-dione maleate salt is an antioxidant which prevents both arachidonic acid-induced and iron-dependent lipid peroxidation[1]. It protects against HR injury in animal organs such as heart, liver and kidney models. These membrane-associating antioxidants are particularly effective in preventing permeability changes in brain microvascular endothelial cells monolayers[2]. A meta-analysis of 24 published seric variables, coming from the same experimental setting, tried to provide a numeric evaluation of the U-74389G efficacy at the same endpoints (Table 1).

The aim of this experimental study was to evaluate the effect of U-74389G in a rat model of HR using blood mean corpuscular volume (MCV) levels.

2. Materials and methods

2.1 Animal preparation

This basic experimental research was licensed by Veterinary Address of East Attiki Prefecture under 3693/12-11-2010 & 14/10-1-2012 decisions. All consumables, equipment and substances used, were a grant of Experimental Research Centre of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki. Accepted standards of humane animal care were adopted for Albino female Wistar rats. 7 days pre-experimental normal housing included *ad libitum* diet in laboratory. Post-experimental awakening and preservation of animals was not permitted even if euthanasia was required. Rats were randomly delivered to four experimental groups by 10 animals in each one, using following protocols of HR: Hypoxia for 45 min followed by reoxygenation for 60 min (group A). Hypoxia for 45 min followed by reoxygenation for 120 min (group B). Hypoxia for 45 min followed by immediate U-74389G intravenous (IV) administration and reoxygenation for 60 min (group C). Hypoxia for 45 min followed by immediate U-74389G IV administration and reoxygenation for 120 min (group D). The molecule U-74389G dosage was 10 mg/Kg body weight of animals.

Prenarcosis preceded of continuous intra-experimental general anesthesia, oxygen supply, electrocardiogram and acidometry[3-6]. Hypoxia was caused by laparotomic clamping inferior aorta over renal arteries with forceps for 45 min. Reoxygenation was induced by removing the clamp and reestablishment the inferior aorta patency. After exclusion of blood flow, the protocol of HR was applied, as described above for each experimental group. U-74389G was administered at the time of reoxygenation through inferior vena cava catheter. The MCV levels were determined at 60th min of reoxygenation (for A and C groups) and at 120th min of reoxygenation (for B and D groups). Fourty female Wistar albino rats were used (mean weight 231.875 g [standard deviation (SD): 36.59703 g], with minimum weight 165 g and maximum weight 320 g. Rats' weight could be potentially a confusing factor, e.g. more obese rats to have higher MCV levels. This assumption was also investigated.

2.2 Control groups

20 control rats of mean weight 252.5 g [SD: 39.31988 g] experienced hypoxia for 45 min followed by reoxygenation.

Group A: Reoxygenation which lasted 60 min concerned 10 controls rats of mean weight 243 g [SD: 45.77724 g] and mean MCV 59.17 fl [SD: 2.730914 fl] (Table 2).

Group B: Reoxygenation which lasted 120 min concerned 10 controls rats of mean weight 262 g [SD: 31.10913 g] and mean MCV 58.81 fl [SD: 1.397975 fl] (Table 2).

Lazaroid (L) group: 20 rats of mean weight 211.25 g [SD: 17.53755 g] experienced hypoxia for 45 min followed by reoxygenation in the

beginning of which 10 mg U-74389G /kg body weight were IV administered.

Group C: Reoxygenation which lasted 60 min concerned 10 L rats of mean weight 212.5 g [SD: 17.83411 g] and mean MCV 61.61 fl [SD: 1.67561 fl] (Table 2).

Group D: Reoxygenation which lasted 120 min concerned 10 L rats of mean weight 210 g [SD: 18.10463 g] and mean MCV 61.22 fl [SD: 1.487578 fl] (Table 2).

2.3 Statistical analysis

Every weight and MCV level group was compared with each other by statistical standard t-tests (Table 3). Any significant difference among MCV levels, was investigated whether owed in probable significant weight correlations. The generalized linear models (glm) with dependant variable the MCV levels were applied. The 3 independent variables were the U-74389G or no drug, the reoxygenation time and both variables in combination. Inserting the rats' weight also as an independent variable at glm analysis, a very significant relation resulted in with MCV levels (p=0.0000), so as to further investigation was needed. The predicted MCV values were calculated for every rat and are depicted at table 5. Every predicted MCV level group was compared with each other by statistical standard t-tests (Table 3). The glm with dependant variable the predicted MCV levels were iterated. The 3 independent variables were again the U-74389G or no drug, the reoxygenation time and both variables in combination.

3. Results

The first glm resulted in: U-74389G administration significantly increased the MCV levels by 2.425 fl [1.235015 fl - 3.614985 fl] (p= 0.0002). This finding was in accordance with the results of standard t-test (p= 0.0026). Reoxygenation time non-significantly increased the MCV levels by 0.3749996 fl [-1.80157 fl - 1.051571 fl] (p= 0.5977), also in accordance with standard t-test (p= 0.1226). However, U-74389G administration and reoxygenation time together significantly increased the MCV levels by 1.251818 fl [0.492508 fl - 2.011128 fl] (p= 0.0019). Reviewing the above and table 3, table 4 sums up concerning the increasing influence of U-74389G in connection with reoxygenation time. The second glm resulted in: U-74389G administration significantly increased the predicted MCV levels by 1.735536 fl [0.9155627 fl - 2.55551 fl] (p= 0.0001). This finding was in accordance with the results of standard t-test (p= 0.0002). Reoxygenation time non-significantly increased the MCV levels by 0.3471077 fl [-1.339179 fl - 0.6449639 fl] (p= 0.4831), also in accordance with standard t-test (p= 0.3375). However, U-74389G administration and reoxygenation time together significantly increased the MCV levels by 0.9657804 fl [0.4538955 fl - 1.477665 fl] (p= 0.0005). Reviewing the above and table 6, the tables 7 and 8 sum up; concerning the increasing influence of U-74389G in connection with reoxygenation time.

Table 1: The U-74389G influence (±SD) on the levels of some seric variables[3] concerning reperfusion (rep) time

Variable	1h rep	p-value	1.5h rep	p-value	2h rep	p-value	interaction of U-74389G and rep	p-value
WBCC	+22.99%±12.45%	0.0914	+30.85%±11.14%	0.0045	+38.70%±17.39%	0.0185	+23.45%±6.28%	0.0004
RBCC	+1.39%±0.71%	0.7161	+0.64%±0.32%	0.8106	-0.10%±0.05%	0.9762	+1.05%±0.53%	0.4911
Hematocrit	+5.58%±3%	0.0852	+4.73%±2.25%	0.0435	+3.89±3.44%	0.2608	+3.16%±1.33%	0.0196
Hemoglobin	+5.2%±2.8%	0.0925	+3.9%±2.1%	0.0604	+2.7%±3.2%	0.3544	+2.5%±1.3%	0.0423
MCH	+1.77%±0.96%	0.0663	+2.40%±0.57%	0.0001	+3.03%+0.71%	0.0003	1.33%+0.36%	0.0005
MCHC[2]	-0.5%±0.74%	0.4820	-0.95%±0.63%	0.1124	-1.4%±1.12%	0.1603	-0.69%±0.37%	0.0655
RbcDW	-6.13%±3.73%	0.0667	-4.96%±2.27%	0.0175	-3.80%±3.07%	0.1383	-2.54%±1.39%	0.679
Platelet count[2]	-17.79%±9.40%	0.0647	-12.83%±5.79%	0.0303	-7.88%±7.83%	0.2939	-6.12%±3.58%	0.0857
Platelet-crit	+3.80%±9.87%	0.6373	+9.23%±6.29%	0.1064	+14.66%±9.03%	0.0833	+6.72%±3.73%	0.0712
PDW	+1.1%±0.88%	0.2368	+1.79%±0.76%	0.0314	+2.49%±1.33%	0.0807	+0.96%±0.46%	0.0396
Glucose	-6.41%±3.50%	0.0663	-8.57%±2.06%	0.0001	-10.74%±2.52%	0.0003	-4.76%±1.28%	0.0005
Creatinine[3]	-15.96%±8.71%	0.0663	-21.02%±5.06%	0.0001	-26.09%±6.12%	0.0003	-11.69%±3.16%	0.0005
Uric acid[4]	+20.86%±14.44%	0.1614	+15.43%±9.10%	0.0960	+10%±12.11%	0.3946	+4.78%±5.64%	0.3873
Total protein	-5.48%±2.99%	0.0663	-7.34%±1.76%	0.0000	-9.20%±2.16%	0.0000	-4.08%±1.10%	0.0000
γGT	+19.35%±18.58%	0.2362	+6.82%±14.89%	0.6442	-5.71%±20.10%	0.7809	+1.23%±9%	0.8877
ALP	+22.66%±12.37%	0.0663	+31.91%±7.69%	0.0001	+41.16%±9.65%	0.0003	+17.75%±4.79%	0.0005
ACP	-112.54%±20.95%	0.0006	-128.45%+14.84%	0.0000	-144.36%±21.62%	0.0000	-74.45%±9.63%	0.0000
CPK	+54.32%±13.75%	0.0012	+35.34%±17.20%	0.0260	+16.37%±30.24%	0.4951	+18.52%±9.44%	0.0770
Sodium	+1.22%±0.66%	0.0707	+0.17%±0.61%	0.7714	-0.87%±1.03%	0.3995	-0.32%±0.36%	0.3693
Chloride	-0.58%±0.77%	0.4533	-0.97%±0.53%	0.0879	-1.36%±0.76%	0.1113	-0.75%±0.38%	0.0159
Calcium	0%±1.75%	1	-0.14%±1.10%	0.8782	-0.28%±1.54%	0.8492	+0.14%±0.64%	0.8245
Phosphorus	-2.23%±5.51%	0.7966	-1.61%±3.32%	0.5789	-1%±4.48%	0.8129	-1.09%±2%	0.5771
Magnesium	+1.33%±3.59%	0.7033	-0.28%±2.75%	0.9171	-1.90%±5.28%	0.7161	+0.36%±4.58%	0.8228
Mean	-0.26%±28.78%	0.2707	-1.90%±30.75%	0.2268	-3.55%±33.95%	0.3011	-1.06%±17.88%	0.2107

Table 2: Weight, MCV levels and Std. Dev. of groups

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
	MCV	59.17 fl	2.730914 fl
B	Weight	262 g	31.10913 g
	MCV	58.81 fl	1.397975 fl
C	Weight	212.5 g	17.83411 g
	MCV	61.61 fl	1.67561 fl
D	Weight	210 g	18.10463 g
	MCV	61.22 fl	1.487578 fl

Table 3: Statistical significance of mean values differences for groups (DG) after statistical paired t test application

DG	Variable	Difference	p-value
A-B	Weight	-19 g	0.2423
	MCV	0.3599998 fl	0.5275
A-C	Weight	30.5 g	0.0674
	MCV	-2.44 fl	0.0777
A-D	Weight	33 g	0.0574
	MCV	-2.05 fl	0.0916
B-C	Weight	49.5 g	0.0019
	MCV	-2.799999 fl	0.0078
B-D	Weight	52 g	0.0004
	MCV	-2.41 fl	0.0105
C-D	Weight	2.5 g	0.7043
	MCV	0.3899994 fl	0.4655

Table 4: The increasing influence of U-74389G in connection with reoxygenation time

Increase	95% c. in.	Reperfusion time	p-values	
			t-test	glm
2.44 fl	0.3113641 fl - 4.568635 fl	1h	0.0777	0.0270
2.425 fl	1.235015 fl - 3.614985 fl	1.5h	0.0026	0.0002
2.41 fl	1.05377 fl - 3.76623 fl	2h	0.0105	0.0015
0.3749996 fl	-1.80157 fl - 1.051571 fl	reoxygenation time	0.5977	0.1226
1.251818 fl	0.492508 fl - 2.011128 fl	interaction		0.0019

Table 5: Predicted MCV levels and Std. Dev. of groups corrected for weights

Group	Mean	Std. Dev.
A	59.73443 fl	1.926011 fl
B	58.93503 fl	1.308871 fl
C	61.01768 fl	0.7503465 fl
D	61.12286 fl	0.7617265 fl

Table 6: Statistical significance of predicted MCV values differences for groups (DG) corrected for weights after statistical paired t test application

DG	Difference	p-value
A-B	0.7993977 fl	0.2423
A-C	-1.283247 fl	0.0674
A-D	-1.388429 fl	0.0574
B-C	-2.082644 fl	0.0019
B-D	-2.187827 fl	0.0004
C-D	-0.1051826 fl	0.7043

Table 7: The predicted increasing influence of U-74389G in connection with reoxygenation time

Increase	95% c. in.	Reperfusion time	p-values	
			t-test	Glm
1.283247 fl	-0.090014 fl - 2.656507 fl	1h	0.0674	0.0653
1.735537 fl	0.9155627 fl - 2.55551 fl	1.5h	0.0002	0.0001
2.187827 fl	1.181713 fl - 3.19394 fl	2h	0.0004	0.0002
0.3471077 fl	-1.339179 fl - 0.6449639 fl	reoxygenation time	0.4831	0.3375
0.9657804 fl	0.4538955 fl - 1.477665 fl	interaction	0.0005	

Table 8: The (%) predicted increasing influence of U-74389G in connection with reoxygenation time

Increase	±SD	Reperfusion time	p-values
+2.12%	±1.16%	1h	0.0663
+2.88%	±0.69%	1.5h	0.0001
+3.64%	±0.85%	2h	0.0003
+0.57%	±0.83%	reoxygenation time	0.4103
+1.60%	±0.43%	interaction	0.0005

4. Discussion

4.1 Hypoxia may influence MCV levels

Yildiz *et al*[7] significantly decreased the blood MCV levels ($p<0.05$) after both testicular torsion rotation and low dose sildenafil citrate administration in male Wistar rats. Nemeth *et al*[8] found higher MCV levels daily for one week in early postoperative period after hind-limb IR (+ allopurinol) than in sham operated and control male rats groups. Berra et *al* [9] enhanced the MCV levels after 14 days experimental infection with Trypanosoma cruzi (Chagas' disease) than 7 days infected and control rats. Putintsev *et al* revealed [10] increased MCV levels in an exacerbated chronic obstructive bronchitis combined with coronary heart disease. Mueller *et al* [11] predicted MCV as an independent factor of severe atherosclerosis in the iliac arterial disease (OR = 2.72 for an increment of 5 fl) and in the femoral-popliteal arterial disease segment (OR = 3.13 for an increment of 5 fl). Higher MCV values were contributed to symptomatic peripheral arterial disease (PAD) lumen reductions > 75% of the proximal segments in patients compared with matched control subjects. Wilke *et al*[12] diagnosed an intestinal leiomyoma that led to chronic anemia and conspicuous MCV of 63 fl and furthermore to angina pectoris. Penix *et al*[13] showed an increased MCV value, normalized by vitamin B_{12} administration in two ischemic cerebral infarctions 16 years after ileal resection for Crohn's disease. Donaldson *et al*[14] induced significant acute decreases in MCV levels, lasting 1-2 days after short-term falls in temperature. Chen *et al*[15] decreased the MCV levels providing regular transfusions and chelation therapy in 75.6% of Diamond Blackfan anemia patients; the 24.4% of which having macrocytic anemia unresponsive to corticosteroids.

This study is the first attempting to relate MCV levels with U-74389G administration.

5. Conclusion

U-74389G administration whether it interacted or not with reoxygenation time has significant increasing short – term effect on recovery pathophysiology of MCV values.

Acknowledgment:

This study was funded by Scholarship by the Experimental Research Centre ELPEN Pharmaceuticals (E.R.C.E), Athens, Greece. The research facilities for this project were provided by the aforementioned institution.

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